I PHYSICAL CHEMICAL STUDIES OF LIVER LACTIC DEHYDROGENASE

II ULTRACENTRIFUGAL STUDIES ON THE STABILITY OF LIPOPROTEINS OF RABBIT SERUM

BY

EDWIN ORLANDO DAVISSON

A. B., Wabash College, 1948

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY IN THE GRADUATE COLLEGE OF THE UNIVERSITY OF ILLINOIS, 1953

URBANA, ILLINOIS

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I PHYSICAL CHEMICAL STUDIES OF LIVER LACTIC DEHYDROGENASE

The enzyme, rat liver lactic dehydrogenase, was isolated and crystallized in cooperation with Dr. D. M. Gibson. The physico-chemical properties of the enzyme were studied by means of sedimentation, diffusion, electrophoresis, viscosity, and density measurements and kinetic studies. The crystalline enzyme appeared to be a stabile homogeneous protein in phosphate buffer.

At infinite dilution $s_{20,w}$ was 7.39×10^{-13} sec., $D_{20,w}$ was 5.8×10^{-7} cm.^{2}/ sec. The partial specific volume was 0.745 ml./g. at 0 degree C. The specific viscosity increment at O degree C. was 0.039. The anhydrous molecular weight was calculated to be 126,000, and the molar frictional ratio was 1.13. This ratio indicated that the shape of the LDH molecule is somewhere between that of a sphere of 34 per cent hydration and an unhydrated ellipsoid with an axial ratio of 3.4.

Upon electrophoresis at 0.8 degrees C. the enzyme migrated as a single substance in the pH range, 5.8 to 7.8, in phosphate buffer. The isoelectric point was 6.3. The mobility toward the anode at pH 7.8 was -0.53×10^{-5} cm.^{2} v.$^{-1}$ sec.$^{-1}$.

An additional development was made on the proposed mechanism of Theorell and Chance for alcohol dehydrogenase. The extension of this to the liver LDH system permitted an analysis of the experimental kinetic data which was in good agreement with the theoretical considerations. The Michaelis-Menten constants and the maximum velocities were shown to be dependent upon the concentration of the second substrate in a predictable manner.

II ULTRACENTRIFUGAL STUDIES ON THE STABILITY OF LIPOPROTEINS OF RABBIT SERUM

Serum lipoproteins, isolated from rabbit, were found to undergo degradative changes upon *in vitro* dialysis against buffered salt solutions. These changes, as studied by ultracentrifugal techniques were manifested by the decrease in the S_f values of the lipoproteins and a release of lipid material.

Isolation and characterization studies were made on serum to identify the materials which stabilize the serum lipoproteins *in vivo*.

Several known compounds were found which prevented the decomposition of the lipoproteins in the dialysis experiments. Ascorbic acid and certain compounds which have a sparing action on ascorbic acid via their capacity to bind metal ions were active stabilizing agents.

Additional studies suggested that the degradative changes were the result of oxidative changes in the lipoprotein by previously formed peroxides.

VITA

Edwin Orlando Davisson was born on January 23, 1923, in Tippecanoe County, Indiana. He graduated from Crawfordsville High School in 1941 and entered Wabash College the following fall. After serving 3 years in the United States Army he returned to Wabash College and received the degree of Bachelor of Arts in June 1948.

He began graduate work at the University of Illinois in September 1948 where he held a teaching assistantship for one year. For the next two years he held an E. I. du Pont de Nemours Special Fellowship. The following year he was appointed to the G. D. Searle Fellowship in Chemistry.

He is a member of Phi Lambda Upsilon, Sigma Xi, Eta Kappa Nu, and Phi Beta Kappa.